Effect of geographical distributions on the nutrient composition, phytochemical profile and antioxidant activity of *Morus nigra*

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Abstract: Recent worldwide inclination for the consumption of natural compounds has extremely augmented the significance of persistent quality of plant materials. Consequently, there is an escalating scientific concern in the impact of geographical distributions of the plants on their chemical constituents, physical characteristics and biological activities. The current study was carried out to see the effect of geographical locations on the nutrient composition, mineral contents, phytochemical profile and free radical scavenging activity of *Morus nigra* fruit. The samples were collected from five different locations of Khyber Pakhtunkhwa, which included districts of D. I. Khan, Karak, Peshawar, Swabi and Swat. The results revealed the considerable impact of geographical locations on the levels of proximate nutrient and selected minerals. Likewise, the concentrations of phenolic, flavonoid, anthocyanin and alkaloidal contents varied significantly ($p<0.05$) with respect to their geographical distributions. The physicochemical characteristic, extraction yields and DPPH scavenging activity of the samples also showed strong link with the sites of their cultivation. The data suggest that geographical distributions affect the levels of phytochemicals and conversely their biological activities. These variations must be taken into consideration while utilizing raw plant materials for industrial applications and traditional therapies.

Keywords: *Morus nigra*, geographical locations, phytochemicals, minerals, DPPH scavenging activity.

INTRODUCTION

The plant species of *Morus* genus are commonly known as mulberries and has the ability to grow under a wide variety of soil types and climatic conditions. The genus is widely distributed in temperate to subtropical areas of northern hemisphere especially in Europe, Asia, North America and Africa (Ercisli and Orhan, 2007; Mazimba *et al*., 2011). Plants of this genus possess a colossal importance in domestic, food, medicinal, economical, clinical and industrial arenas. The fruit juice has been used as folk remedies for tumors, asthma, cold, cough, diarrhea, dyspepsia, edema, fever, headache, hypertension and wounds. Barks and roots have purgative, anthelminthic and astringent properties, while leaves play a vital role in rearing silkworm, *Bombyx mori* (Absar *et al*., 2005; Venkatesh and Chauhan, 2008). Mulberry leaves are also used as cattle fodder and known to enhance milk yield (Dillard and German, 2000). Cyanidin-3-rutinoside and cyanidin-3-glucoside found in mulberries are reported to have an inhibitory effect on incursion and migration of lung cancer cells (Chen *et al*., 2006).

Morus nigra Linn. is one of the most common mulberry specie. The general name of the plant is black mulberry. It is widely cultivated in Asia and Europe (Ercisli and Orhan, 2007; Hojjatpanah *et al*., 2011). The fruits are purple to black in color and famous for their nutritional qualities, deliciousness and unique flavor (Koyuncu *et al*., 2004; Özgen *et al*., 2009; Venkatesh and Chauhan, 2008). These are consumed both fresh and in dry state, and are widely used for making juices, beverages, wines, marmalades, jams, jellies and food colors (Hojjatpanah *et al*., 2011). The fruit contains calcium, phosphorus, iron, thiamine, nicotinic acid, riboflavin and ascorbic acid (Nitra *et al*., 2007; Venkatesh and Chauhan, 2008). The root barks, twigs and fruits of *Morus nigra* are traditionally prescribed to treat cough, asthma, chest complaints and rheumatism (Khalid *et al*., 2011; Mazimba *et al*., 2011). The fruits are especially useful in the treatment of mouth lesions, sore throat, fever, dyspepsia and melancholia (Iqbal *et al*., 2010). It has good impact on blood glucose level (Darias-Martin *et al*., 2003) and can also control blood cancer (Ahmad *et al*., 1985). The decoction of the leaves has blood-purifying properties (Mazimba *et al*., 2011). The root extracts of the plant exhibited ability to reduce blood sugar in diabetic patients (Ahmad *et al*., 1985; Venkatesh and Chauhan, 2008). The root extracts contains deoxyjirimycin, an alkaloid, which is considered to be effective against HIV (Venkatesh and Chauhan, 2008).

Phytochemicals are getting more and more attention than ever as these have now numerous applications in industries such as food, pharmaceutical, nutraceutical and cosmetic etc. Previous R & D work has revealed significant effect of geographic locations on the types and levels of phytochemicals in other plants **(**Khan *et al*., 2011). The ensuing variations in biological properties affect the effectiveness of the raw plant materials as well as the quality of finished products (Badoni *et al*., 2009;

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Gupta *et al*., 2011; Ullah *et al*., 2012; Yang *et al*., 2005). Because of the inconsistent efficacy of the products, the manufacturers may face shattered credibility among the consumers.

No information is available on the correlation of geographical origins on the phytochemical profile and antioxidant activities of the *Morus nigra*. The current study was undertaken to find the effect of geographical distributions of the plant on the proximate nutrients, minerals, phytochemicals, extraction yield and scavenging activities.

MATERIALS AND METHODS

Plant materials

The fruits were harvested from the cultivated plants of *Morus nigra*, from Swat, Peshawar, Karak, Swabi and D. I. Khan in the months of April and May, 2012.Fruits were wrapped in dark plastic bags to protect them from direct sunlight. Samples were kept immediately at 4°C and transferred to laboratory.

Samples preparation

Fruits were cleaned and surface moisture removed with a parched cloth. The comestible portions were detached and dried in an oven at 45°C. The dried materials were pulverized in a grinder (Retch Muhle-Germany), passed through the 30mesh sieve and packed in clear polyethylene pouches. The samples were sealed using an electric sealer (PFS 300, Japan). For determination of titratable acidity and pH fresh fruits were used.

Determination of pH and titratable acidity

For the estimation of titratable acidity and pH, the fruits were squeezed and filtered. Fifteen ml of the juice was diluted with 35ml of distilled water in a 50ml flask. The solutions were stirred at 200 rpm on magnetic stirrer for half an hour at 25°C and then filtered. The pH values were determined in triplicate using a Microcomputer pH Vision Datalogger (6091, JENCO electronic Ltd., China) at room temperature. Three point calibrations were performed with pH 7.0, 4.0 and 2.0 buffers (Fischer Scientific). For the determination of titratable acidity, 5 ml of the solution was taken in a 25ml flask and then2 to 3 drops of phenolphthalein (1%) were added. Subsequently this was titrated with 0.1N sodium hydroxide.

Preparation of extracts

The samples (50 g, each) of *Morus nigra* were extracted in methanol (3×150ml) with a Soxhlet extractor. All extracts were sifted by Whatman No.1 filter paper, combined and concentrated to dryness in a rotary evaporator at 45°C. The weights of all dry extracts were determined. Extraction yields were estimated by deducting the dry weight of plant residue after extraction from the weight of the initial plant materials. The extracts were kept in a refrigerator at 4°C till further processing.

Proximate analysis

The plant samples were assessed for moisture, ash, crude fat, crude protein and fiber on a dry weight basis, according to AOAC (1990). The moisture was measured in a drying oven at 105°C until constant weight. Analysis of crude fat was done in a Soxtec system HT (Tecator) using petroleum ether (bp. 40-60°C) Determination of crude protein (% N x 6.25) was accomplished by employing micro-Kjeldahl method. Crude fiber was estimated by treating the samples with acid and alkali using Fibertec system (Tecator) and ash contents measured by heating the samples at 550°C. Carbohydrates (%) contents were estimated by using a difference method, by deducting the sum of the percent of moisture, fat, protein, ash and fiber from 100. The calorific value was measured by multiplying the values of carbohydrate, lipid, and protein with the factors 4, 9 and 4, respectively. Then the products were summed and expressed in kcal per 100 g. The assessment of ascorbic acid was carried out with 2,6-dichlorophenol-indophenol using titration method.

Mineral analysis

The dried samples of plants were evaluated by wet digestion method using a combination of nitric and perchloric acid. Each analysis was done in triplicate. One gram of each sample was digested with a 20ml of diacid mixture (HNO_3: $HClO_4$, 5: 1, v/v) in a fume cupboard, heating initially at 80°C and then temperature was gradually increased to 250°C. After complete digestion, each sample was heated to near dryness (approximately 1-2ml). The digested samples were cooled and transferred to a flask. The volume was brought up to 50ml using double distilled deionized water and samples were then filtered through Whatman No. 42 filter paper. Analyses were done using atomic absorption spectrophotometer (Perkin Elmer, USA), flame photometer (Jenway, England) and spectrophotometer (Shimadzu, Kyoto, Japan).The phenolic contents of fruit samples were appraised by using the Folin-Ciocalteau colorimetric assay (Khattak, 2012). In a test tube, 200µl of filtered methanolic extract was added to 4ml of 2% aqueous sodium carbonate solution and thoroughly mixed. Afterward 200µl of 50% Folin-Ciocalteau reagent was added to the solution. The mixture was allowed to stand for 1 hour and the absorbance of green-blue complex was taken at 750nm in a spectrophotometer against blank. The results were described as milligram of gallic acid equivalents per gram (mg/100g) of the dry extract.

Determination of anthocyanin

The total monomeric anthocyanin content was evaluated by a pH differential method (Giusti and Wrolstad, 2001). Absorbance was recorded with a UV-visible spectrophotometer at 510 and 700nm at pH 1.0 and 4.5 employing the following equation.

A= (A510nm−A700nm) pH1.0-(A510nm−A700nm) pH4.5

Data were calculated with the extinction coefficient for cyanidin-3-glucoside (ε=29,600) and the results are described as mg cyanidin-3-glucoside per 100g dry weight.

Determination of alkaloids

The alkaloidal contents were determined using the method of Harborne (1973). Five gram of powdered fruit sample was taken in a 250 ml beaker and to this 200ml of 10% acetic acid in ethanol was added. The solution was covered and incubated at room temperature for 3 hours. Afterward, the mixture was filtered and concentrated to one quarter of the original volume on a water bath. Concentrated ammonium hydroxide was drop-wise added to the extract and the precipitates were collected. These were afterward washed properly with dilute ammonium hydroxide and filtered. The residues were dried and weighed.

Estimation of flavonoids

Flavonoids contents were assessed by employing the method of Aiyegoro and Okoh (2010). One ml of the fruit extract was mixed with 0.2ml of 10% aluminum chloride, 0.2ml of 1M potassium acetate, 3ml of methanol and 5.6ml of distilled water. The mixture was kept at room temperature for half an hour. The absorbance of the mixture was noted at 420 nm. Quercetin was employed as standard (0-1mg/ml). Concentration levels of flavonoids were calculated from the standard curve and reported as mg/100g quercetin equivalents.

Determination of DPPH radical scavenging activity

The scavenging activity of the fruit samples was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Khattak, 2012). Two ml of DPPH radical in methanol solution (60µM) was mixed with eighty µl of the sample and shaken appropriately. The solution was incubated in dark at 37°C for an hour and then its absorbance was measured at 517nm. The absorbance of corresponding blank (control) was also noted. The results were expressed as EC_{50} values. EC_{50} value is defined as the extract concentration at which DPPH radicals were reduced by 50%and it is estimated from the linear regression analysis.

STATISTICAL ANALYSIS

All determinations were based on triplicate measurements and results were recorded as means ± standard deviations. The data were evaluated by one-way ANOVA and least significant difference tests for the mean differences for all the parameters. Statistical significance was proclaimed at $p<0.05$.

RESULTS

Geographic locations of the plants may influence the types and levels of their phytochemicals, which resultantly affect the quality of the produces. The present study is a comparative analysis of phytochemicals, proximate nutrients, mineral contents and scavenging activity of *Morus nigra* fruits collected from five districts of Khyber Pakhtunkhwa.

The proximate compositions of black mulberry fruits collected from various geographic locations are given in table 1. The moisture content of the fruits was in the range of 8.9 to 12.3%. The highest moisture value was recorded for the sample collected from Swat and lowest for the *sample acquired* from Swabi. The ash contents were found different at the significance level of $p<0.05$ for all the tested fruits. The ash values ranged between 7.0 (sample from Swat) to 9.8% (sample from Peshawar). The analysis of the crude fats, indicated the concentrations between 2.7 to 4.7%. The sample acquired from Swabi showed the maximum value. In case of protein contents, the samples collected from D. I. Khan showed the highest value (13.1%), followed by that of the samples collected from Peshawar (11.1%), Swat (9.4%), Karak (9.3%) and Swabi (8.6%). The fibre contents of the fruits varied significantly ($p<0.05$) with respect to collection points. The fruits collected from Swabi showed highest fibre content (14.4%). The carbohydrate contents depicted significant variations in their concentrations ($p<0.05$) with reference to sampling sites. Highest value was observed for the sample collected from Swat. The calorific values, calculated on a dry weight basis, ranged between 278.8 to 297.1 kcal/100g. However, these values were found statistically ($p>0.05$) the same for all the samples.

The mineral composition of black mulberry fruits is shown in table 2. All the selected elements (K, P, Ca, Na, Mg, Fe, Zn and Mn) were detected in all the tested fruits but their concentrations were found significantly different. Phosphorus and potassium were the predominant elements. The levels of these elements were found changed for the tested samples at the significance level of $p<0.05$. Similarly, the concentrations of calcium varied from 337 to 502mg/100g. The sodium content of *Morus nigra* fruits were found statistically ($p>0.05$) the same for all samples, except for the fruit collected from Swabi. The magnesium contents ranged between 255 to 386mg/100g. However, these were found statistically ($p>0.05$) same for the samples collected form Peshawar, Swabi and D. I. Khan. The data showed that iron and manganese content ranged between 22 to 43mg/100g and 9 to 19mg/100 g, respectively. Both the elements showed significant variations ($p<0.05$) with respect to geographic locations of the samples. The zinc contents ranged from 35 to 62mg/100g.

The ascorbic acid, phenolic, flavonoid, anthocyanin and alkaloidal contents of the fruits of *Morus nigra* were determined and results are presented in table 3. The vitamin C contents of fruit samples ranged from 19.3 to

32.7mg/100g. The data showed significant effect ($p< 0.05$) of collection points on vitamin C levels. Maximum value was recorded for the sample collected from Swabi, while lowest for the samples of Swat and Peshawar. A study performed by Iqbal *et al*. (2010) depicted 32mg/100 g vitamin C content in fresh black mulberry fruit. The phenolic content of the *Morus nigra* fruits ranged between 558.0 to 1090.7mg of gallic acid equivalent (GAE) per 100g. Earlier, Khalid *et al*. (2011) reported 2050 µg/g phenolic contents in the fresh juice of black mulberry, whereas a study conducted by Özgen *et al*. (2009) showed 2737 µg/g phenolics in *Morus nigra* fruits. Mahmood *et al*. (2012) reported 575, 1722 and 2287 mg/100 g phenolics in unripen, semi ripened and fully ripened samples of *Morus nigra*, respectively. The highest quantity of total phenols was found in the *sample collected from D. I. Khan.* Flavonoid contents of the fruits of the samples varied from 63.7 to 244.0 mg/100g. Previously, Mahmood *et al*. (2012) reported 245, 706 and 1021mg/100g flavonoids in unripen, semi ripened and fully ripened samples of *Morus nigra*, respectively. Yang *et al*. (2010) reported 0.39% total flavonoids in *Morus alba* fruit. Highest content was recorded for sample collected from Swat. The samples obtained from Karak and Swabi with 135.0 and 147.7mg/100g flavonoids were found statistically equal. The analysis of anthocyanins depicted significant variations with reference to the geographical origins of the plant. The anthocyanins ranged from 67.0 to 346.3 mg of cyanidin 3-glucoside equivalent per 100g on dry weight basis. Highest content were observed for sample picked from Swat and lowest for sample of Peshawar. Earlier research conducted by Ercisli *et al*. (2010) showed that the black mulberry contained 719 µg/g of anthocyanin content on fresh mass basis. While, a study carried out on Turkish mulberry species (Özgen *et al*., 2009) indicated that black mulberry had the highest amount of anthocyanins (571µg/g). The alkaloids ranged between 404.0 to 648.3 mg/100 g on dry weight basis. Previously, Imran *et al*. (2010) analysed the fruits of four *Morus* species and found that *Morus nigra* contained 630mg/100 g of alkaloid contents.

The pH values and titratable acidity of the fruit samples are presented in fig. 1. The pH values of the selected samples were in the range of 3.4 to 4.9. The sample collected from D. I. Khan showed the highest pH, followed by that of the Peshawar and Karak. The pH values (3.52-5.60) reported earlier by Ercisli and Orhan (2007) are in good agreement with the present findings. The titratable acidity of the tested samples ranged from 0.9 to 1.7%. Significant variations ($p<0.05$) were noted for acidity values for all samples except for the fruit grown in Swat and Peshawar. These results are in line with previous findings reported by Imran *et al*. (2010), which showed titratable acidity ranging from 0.84 to 2.00% for the fruits of *Morus* species.

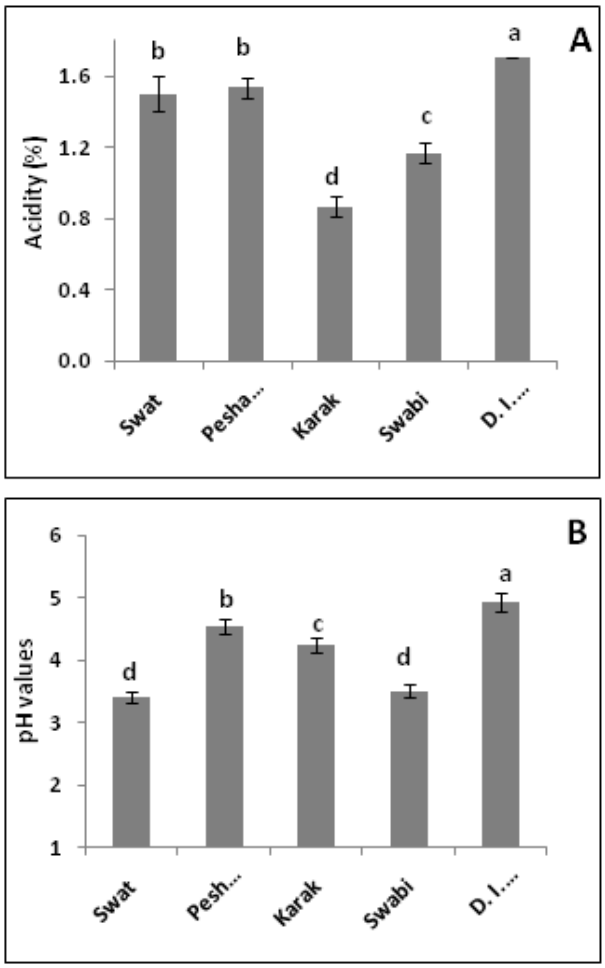

Fig. 1: Titratable acidity (A) and pH values (B) of *Morus nigra* fruit collected from different geographic locations of Khyber Pakhtunkhwa. The values are means of triplicate determinations (n=3) ± standard deviations. The vertical bars represent the standard deviation for each data point. Values with different superscript letters are significantly different ($p<0.05$).

The dry weight yields of the methanol extracts of the *Morus nigra* fruits were determined and presented in fig. 2. The extraction yields ranged between 8.7 to 26.7%. The free radical scavenging activity of the methanolic extracts of the plants was analyzed by using DPPH radical. The results are reported as EC_{50} values (µg/ml) and shown in fig. 3. Low EC_{50} is the sign of high DPPH scavenging activity. The data showed significant variations in EC_{50} values regarding geographic origins of the plants. The values ranged from46.7 to 458.4µg/ml. Highest DPPH scavenging activity was observed for the sample collected from D. I. Khan, followed by that of the Swabi (53.7µg/ml), Swat (96.3µg/ml), Karak (162.1µg/ml) and Peshawar (458.4µg/ml).

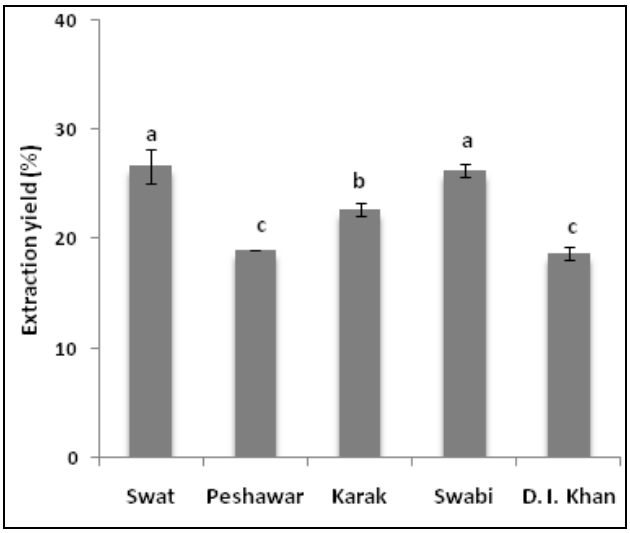

Fig. 2: The extraction yields of *Morus nigra* fruit collected from different geographic locations of Khyber Pakhtunkhwa. Values are means of triplicate determinations (n=3) ± standard deviations. The vertical bars represent the standard deviation for each data point. Values with different superscript letters are significantly different ($p<0.05$).

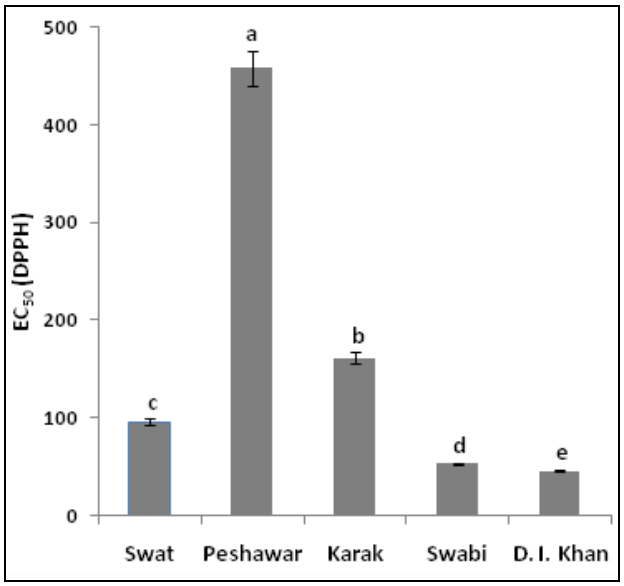

Fig. 3: The EC_{50} value (µg/ml) of the DPPH scavenging activity of *Morus nigra* fruit collected from different geographic locations of Khyber Pakhtunkhwa. Values are means of triplicate determinations (n=3) ± standard deviations. The vertical bars represent the standard deviation for each data point. Values with different superscript letters are significantly different ($p<0.05$).

DISCUSSION

Statistical analysis of the current data showed significant effect of sampling sites on the proximate constituents and energy values of *Morus nigra* fruits. There is no information in the literature on the influence of geographic locations on the proximate nutrients of the plant. However in contrast to our findings, a study conducted by Inekwe *et al*. (2012) on *Jatropha curcas* seeds, collected from India, Kaduna (Nigeria) and Edo (Nigeria) showed that moisture, ash and fiber contents were not affected by the geographical locations. However the researchers found statistically significant variations in the concentrations of lipid, protein and carbohydrate contents with respect to collection sites. Similarly, Elfeel (2010) evaluated the seed kernel of *Balanites aegyptiaca* var. *aegyptiaca* of three separate ecological zones across Sudan and found significant variations in the concentrations of oil and protein contents. Badoni *et al*. (2009) reported that the concentrations of essential oils of *Artemisia nilagirica* varied significantly with the altitudes of collection sites. Though no literature is available to correlate the effect of geographical distribution on the mineral constituents of the plant, however, a study accomplished by Elfeel (2010) showed significant variations in the concentrations of nitrogen, phosphorus, iron, calcium, potassium and magnesium contents in seed kernels of *Balanites aegyptiaca* var. *aegyptiaca* from three separate ecological zones across Sudan.

Effect of geographic location on vitamin C content is not known for *Morus nigra* fruits, however, a study executed by Gull *et al*. (2012) revealed that the vitamin C content of guava fruits significantly changed with geographic distributions and ripening stages of the plant. The data of the present study revealed that geographic locations of the plants had significant ($p<0.05$) effect on the phenolic, flavonoids and alkaloidal contents. The analysis of phytochemicals of *Tribulus terrestris* fruits (Ashwani and Ashish, 2012) from six different districts of North India showed substantial variations in the levels of saponins, alkaloids and flavonoids. Similarly, chemically investigation performed by Gull *et al*. (2012) on the guava fruits, collected during different stages of ripening from Faisalabad, Islamabad and Bhakkar showed that phenolic and flavonoid contents significantly varied both with geographic locations and ripening phases. A scientific investigation on the phytochemicals of *Cassia occidentalis* revealed that the plant collected from Ivory Coast, Africa contained a little amount of saponins and had no triterpenes, tannins, alkaloids, sterols, flavonoids and quinines, while a large quantity of alkaloids was observed in the leaves, stems and fruits of the same plants collected from Ethiopia (Yadav *et al*., 2010). Muraina *et al*. (2008) worked on the leaves of *Anoigeissus leiocarpus* picked from two different geographical locations in Nigeria and explored that there were ample variations in the phytochemical constituents between the two plant extracts. Yang *et al*. (2005) estimated different phytochemicals (1,5,8-trihydroxy-3-methoxyxanthone, 1,8-dihydroxy-3,7-dimethoxyxanthone, 1,8-dihydroxy-3,5-dimethoxyxanthone, swertiamarin, mangiferin,

Table 1: Proximate composition of *Morus nigra* fruit collected from different geographic locations of Khyber Pakhtunkhwa

Nutrients	Locations				
	Swat	Peshawar	Karak	Swabi	D. I. Khan
Moisture (%)	12.3±0.9^{a}	11.6±0.6^{a}	10.1±0.7^{b}	8.9±0.9^{b}	9.6±0.4^{b}
Crude protein (%)	9.4±0.3^{c}	11.1±0.5^{b}	9.3±0.2^{c}	8.6±0.9^{c}	13.1±0.8^{a}
Crude fat (%)	2.7±0.2^{c}	3.2±0.2^{b}	4.1±0.5^{a}	4.7±0.5^{a}	2.7±0.1^{c}
Ash (%)	7.0±0.3^{e}	9.8±0.4^{a}	7.9±0.3^{d}	8.3±0.1^{c}	9.1±0.4^{b}
Crude fiber (%)	13.0±0.6^{b}	13.0±0.6^{b}	14.2±0.4^{a}	14.4±0.4^{a}	11.6±0.1^{c}
Carbohydrate (%)	57.0±0.3^{a}	51.4±0.4^{c}	54.3±0.7^{b}	54.8±1.3^{b}	53.8±1.6^{b}
Energy (kcal/100g)	289.9±12^{a}	278.8±17^{a}	291.7±14^{a}	297.1±10^{a}	292.5±8^{a}

Table 2: Mineral composition (mg/100g) of *Morus nigra* fruit collected from different geographic locations of Khyber Pakhtunkhwa

Minerals	Locations				
	Swat	Peshawar	Karak	Swabi	D. I. Khan
Ca	455±25^{b}	337±10^{c}	501±26^{a}	461±6^{b}	502±12^{a}
Na	302±4^{a}	287±12^{a}	300±7^{a}	266±6^{b}	283±9^{a}
Mg	255±3^{c}	386±17^{a}	267±2^{b}	367±12^{a}	352±19^{a}
Fe	29±0.2^{d}	22±0.1^{e}	43±0.2^{a}	34±0.0^{c}	41±0.3^{b}
Mn	11±0.1^{d}	9±0.0^{e}	14±0.0^{c}	19±0.2^{a}	16±0.1^{b}
Zn	45±0.2^{c}	35±0.2^{d}	35±0.1^{d}	53±0.2^{b}	62±0.3^{a}
K	2234±67^{a}	1819±35^{c}	1736±19^{d}	2123±100^{b}	1452±56^{e}
P	2520±88^{a}	1639±45^{e}	2185±86^{b}	1932±66^{c}	1765±75^{d}

The values presented in this table are on dry weight basis. Values are means ± standard deviations of three determinations. Means with different superscript letters within the same row are significantly different ($p<0.05$).

Table 3: Phytochemical profile (mg/100g) of *Morus nigra* fruit collected from different geographic locations of Khyber Pakhtunkhwa

Phytochemicals	Locations				
	Swat	Peshawar	Karak	Swabi	D. I. Khan
Phenolics	880.3±17.0^{d}	558.0±34.2^{e}	995.0±15.7^{b}	930.0±5.6^{c}	1090.7±50.6^{a}
Anthocyanins	346.3±5.1^{a}	67.0±4.6^{e}	263.7±12.2^{b}	205.7±7.5^{c}	159.7±7.0^{d}
Flavonoid	244.0±10.1^{a}	63.7±6.8^{d}	135.0±6.1^{b}	147.7±6.4^{b}	93.3±7.5^{c}
Alkaloids	446.3±10.1^{d}	404.0±16.4^{e}	571.7±12.9^{b}	648.3±11.0^{a}	520.0±17.3^{c}
Ascorbic acid	19.3±0.6^{c}	19.3±1.5^{c}	24.3±2.1^{b}	32.7±1.5^{a}	26.3±0.6^{b}

Values are means ± standard deviations of three determinations. Means with different superscript letters within the same row are significantly different ($p<0.05$).

swertisin and oleanolic acid) in the samples of *Swertia mussotii* and reported considerable variations in the concentrations of these with respect to the altitudes.

The present study explored that all the tested samples have significant variations ($p<0.05$) in the extraction yields with reference to their collection locations. This is in agreement with the results of recent study (Gull *et al*., 2012), which indicated that the yields of methanolic extracts of guava fruits were strongly affected by the geographic locations. It was clear from the data that geographical locations have substantial effect ($p<0.05$) on the DPPH scavenging activity. Previously, a study conducted by Gull *et al*. (2012) showed that DPPH radical scavenging activity of guava fruit was significantly affected by geographic locations (Faisalabad, Islamabad and Bhakkar). Muraina *et al*. (2008) also reported that antioxidants, antimicrobial and cytotoxi activities of *Anoigeissus leiocarpus* leaves were strongly affected origins. A study undertaken by Ullah *et al*. (2012) showed the substantial impact of geographical locations on the antibacterial activities of *Mentha spicata* L.

In short, the current data clearly revealed that phytochemicals and nutrients are strongly influenced by the geographic sites of the plants and these variations can consequently ensure significant changes in the biological activities.

CONCLUSION

In the current study, the results showed significant variations in the concentration levels of proximate nutrients, minerals and selected phytochemicals of *Morus nigra* seeds with reference to the area, where they were collected form. The physicochemical and scavenging properties of the plant also exhibited a strong link with its surroundings and ecological distributions. However, no significant conclusion could be derived regarding the direct and sole effect of geographic location on the chemical constituents and biological activities of the plant, as these are also affected by soil types, growing conditions, nutrient availability, fertilizer applications, plant's age, variety, climate and treatments etc.

Variations in the levels of active ingredients and biological properties of raw plant materials may consequently affect the effectiveness of traditional plant-based therapies and quality of the finished products, to which they are added. Therefore, these variations must be taken into consideration while utilizing raw plant materials for industrial applications and traditional therapies.

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